PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Epidemiology and Genetics of Common Mental Disorders in the general population: the PEGASUS-Murcia project
AUTHORS	Navarro-Mateu, Fernando; Tormo, Mª José; Vilagut, Gemma; Alonso, Jordi; Ruiz-Merino, Guadalupe; Escámez, Teresa; Salmerón, Diego; Júdez, Javier; Martínez, Salvador; Navarro, Carmen

VERSION 1 - REVIEW

REVIEWER	Toyokawa, Satoshi The University of Tokyo
REVIEW RETURNED	23-Oct-2013

The reviewer completed the checklist but made no further comments.

REVIEWER	Uddin, Monica Wayne State University School of Medicine
REVIEW RETURNED	23-Oct-2013

GENERAL COMMENTS	The manuscript by Navarro-Mateu et al. describe a comprehensive protocol designed to collect survey and biologic data from non-institutionalized adults in the Murcia region of Spain, with a goal of assessing the prevalence of common mental disorders in this region and identifying risk and protective factors for these disorders (both biologic and environmental). This is an ambitious project with overall laudable goals; however, the paper as written suffers from a number of shortcomings in the biologic aspects of this study. Despite a sizeable focus within the paper on the potential for biologic (genetic, epigenetic, and gene expression assays) within this protocol, the nature of the biological sample is not specified in the methods section. Elsewhere (in the abstract, and in the discussion) it says there will be 2 buccal samples—this should be stated in the methods. However, it is highly unusual to collect buccal samples for RNA analysis in psychiatric studies; there thus needs to be some up-front rationale provided for this choice of tissue. More concerning, however, is that elsewhere in the methods section there is reference to the use of kits designed specifically for blood (i.e. the QIAamp DNA Blood Mini Kit). None of this is clearly explained. Swabs (mentioned earlier in the methods section) can't be used to collect blood; and if blood is to be collected, you need trained clinicians to do this. Who is the personnel that will conduct this collection? The methods state that “specially trained technicians” will be used to “monitor the specimen collection by donor” and to “perform sample manipulations.” At what point will people be consented—at the end of the survey conducted by the company with whom the authors have a contract? Is there a

	separate date set for collection of the biospecimen? How will this be operationalized? If the authors plan to collect blood, is it realistic to expect participants to do this without some sort of incentive (which the methods state there will not be, at least for the survey section)? I also recommend that the authors have their paper reviewed closely by a native English speaker. There were several instances of sentences that did not make sense (e.g. “An example of the former is a case-control study of the GxE interactions, involving 5-HTTLPR polymorphisms, designed to analyse the impact of an earthquake in the mental health of the population exposed have been recently granted.”).

VERSION 1 – AUTHOR RESPONSE

Comments to Reviewers
NOTE: Lines and pages correspond to the marked-up manuscript.
We would like to thank your constructive comments to our manuscript.
1. The nature of the biological sample?
Reviewer’s comments: “… This is an ambitious project with overall laudable goals; however, the paper as written suffers from a number of shortcomings in the biologic aspects of this study. Despite a sizeable focus within the paper on the potential for biologic (genetic, epigenetic, and gene expression assays) within this protocol, the nature of the biological sample is not specified in the methods section. Elsewhere (in the abstract, and in the discussion) it says there will be 2 buccal samples—this should be stated in the methods. However, it is highly unusual to collect buccal samples for RNA analysis in psychiatric studies; there thus needs to be some up-front rationale provided for this choice of tissue.”
Only buccal cells have been obtained from interviewees by scraping the oral mucosa. This strategy, although still unusual in psychiatric studies, is increasingly considered as an easy, save, inexpensive and non-invasive method to be added in general population surveys to obtain DNA and/or mRNA (Lee YH, Wong DT. Saliva: an emerging biofluid for early detection of diseases. Am J Dent. 2009;22:241-248; Yan W, Apweiler R, Balgley BM et al. Systematic comparison of the human saliva and plasma proteomes. Proteomics Clin Appl. 2009;3:116-134; Ballantyne J. Validity of messenger RNA expression analyses of human saliva. Clin Cancer Res. 2007;13:1350; Park NJ, Zhou X, Yu T et al. Characterization of salivary RNA by cDNA library analysis. Arch Oral Biol. 2007;52:30-35; Lee YH, Zhou H, Reiss JK et al. Direct saliva transcriptome analysis. Clin Chem. 2011;57:1295-1302; and Palanisamy V, Wong DT. Transcriptomic analyses of saliva. Methods Mol Biol. 2010;666:43-51. doi: 10.1007/978-1-60761-820-1_4.:43-51). The nature of the biological sample has been described in the methods section (pages: 15-16; lines 381-394 of the tracked version) and the rationale for this choice of tissue has been introduced and referenced in the introduction section (page: 6; lines 138-145).

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2. Kits designed specifically for blood.
Reviewer’s comments: “More concerning, however, is that elsewhere in the methods section there is reference to the use of kits designed specifically for blood (i.e. the QIAamp DNA Blood Mini Kit). None of this is clearly explained. Swabs (mentioned earlier in the methods section) can't be used to collect blood; and if blood is to be collected, you need trained clinicians to do this. Who is the personnel that will conduct this collection? The methods state that “specially trained technicians” will be used to “monitor the specimen collection by donor” and to “perform sample manipulations.” At what point will people be consented—at the end of the survey conducted by the company with whom the authors have a contract? Is there a separate date set for collection of the biospecimen? How will this be operationalized? If the authors plan to collect blood, is it realistic to expect participants to do this without some sort of incentive (which the methods state there will not be, at least for the survey section)?”

The biological samples obtained are only buccal cells from the oral epithelium by scraping it with swabs, not blood in any case. This has been clarified in the method section (page 15; lines 369-381). Although the kits are named “for blood” (i.e. the QIAamp DNA Blood Mini Kit), they can be used for other tissues following the manufacturer’s instructions and this has been clarified in the draft (page 17; lines 411-416). Specifically, QIAamp DNA Blood Mini Kits provide fast and easy methods for purification of total DNA for reliable PCR and Southern blotting from whole human blood, buffy coat, cultured cells, lymphocytes; plasma, serum, body fluids, and buccal swabs. There is a specific protocol for DNA purification from buccal swabs (page 36 of the Handbook; http://www.qiagen.com/products/catalog/sample-technologies/dna-sample-technologies/genomic-dna/qiaamp-dna-blood-mini-kit#resources).
The biological samples have been obtained by the interviewers on completion of the interview and only after signing an informed consent. They were trained by one of our colleagues (pages 15-16; lines 381-394). The trained technicians mentioned in the text are those from the BIOBANC-MUR who will analyze the samples. This has been clarified in the manuscript (page 17; line 425).

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3. A native English speaker revision.
Reviewer’s comments: “I also recommend that the authors have their paper reviewed closely by a native English speaker…”
A native English speaker has carefully reviewed the manuscript and corrections have been highlighted using the track changes mode in MS Word and/or by colored text in the manuscript.